Chapter 11

Histopathological Evaluation of Combined Root Canal Filling: An Animal Study

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《Abstract》

Since the study of root canal filling combined with gutta-percha points and endodontic cement by Dixon and Rickert, many other studies have made use of radiographic evaluation. However, histopatholgical evaluations are infrequent and often incomplete in terms of findings. In this chapter we report the results of our examination using gutta-percha points and a new endodontic cement called KEZ with powder components: 40% zinc oxide, 20% calcium hydroxide, 20% iodoform, and 20% resin; and liquid components: 90.5% eugenol, 9% resin and 0.5% setting agent. One hundred dog teeth were perforated using a rotary cutting instrument. The exposed pulp chamber was left opened for 30 days. Rotary reamers were used to perforate the infected root canals at the apical portion. They were cleaned with sodium hypochlorite and saline solution. Fifty canals were filled with combined gutta percha and KEZ. In the other fifty, no root canal filling was used and the teeth were restored with amalgam. Three months later the dogs were sacrificed and specimens for histopathological evaluation were prepared. Resuts were as follows. Root canals filled with a combination of gutta-percha and KEZ manifested proper sealing of the apical apex, appropriate radiopacity, and reliable healing of the apical periodontal tissues. In unfilled root canals, no reparative change occurred. Moreover, when root canals were filled, alveolar bone regeneration, closure of the root apex and cicatrization of the periodontal tissue in deep layers occurred. In addition, sealing of the artificially-made apical foramen was observed in some cases. When canals were filled, both inflammatory cell infiltration and suppuration tended to decrease. Alveolar bone resorption and closure of the root apex occurred as a consequence of new cementum or bone deposition.

Introduction

Gutta-percha points combined with endodontic cement is a method of filling intracanal cavities where a solid gutta-percha point is inserted into the root canal and filler is used to fill up the gaps and seal the canal. It is inevitable to completely seal the canal using only gutta-percha points without filler. This technique is widely used because it provides retention of the gutta-percha points inside the canal using a satisfactory endodontic sealer. This combined use of root canal filling began in the form of gutta-percha points and phosphoric acid cement [1]. The initial histopathologic research regarding root canal filling which used endodontic cement and gutta-percha point was done by Dixon and Rickert in 1938 [2]. The search for the theoretical basis of combined root canal filling material started at that time; however, the study concerning root canal filling is still insufficient, and studies using dog teeth have not yet been reported. The combination of endodontic cement and gutta-percha point for root canal filling has been examined by many researchers, but most studies were done in a clinical setting with radiographic examination, and those done for histopathologic studies have been very few [3-6]. In order to understand the actual condition concerning the healing progress of the root apex using a combined method for root canal filling, detailed examination is not only done through radiographs; histopathological examination is also necessary. However, sufficient a theoretical basis cannot be obtained from recent studies, which are limited to studies like that by Davis (1971), who reported the use of the combination of cement and gutta-percha point in 32 dog canines as prescribed by Rickert [7]; by Baker (1972) [8], in which root canal filling depended on the use of combined zinc oxide eugenol cement and gutta-percha points; and Grossman's prescription of cement and gutta-percha as used by Binnie (1973) [9] in a study of dog teeth after pulp extirpation. Nevertheless, in each case, the study did not show clinical infection after the root canal had been anesthesized, extirpated and obturated. Furthermore, in experiments using dog teeth, the majority did root canal filling in canal access not penetrating the root apex. The healing progress of the root apex after root canal filling of this kind has not been reported yet. Even if an assumption of satisfactory results could be obtained, it is necessary to do further examinations of the root apex. To consider this kind of circumstance, endodontic cement KEZ combined with gutta-percha point was used as root canal filling in 50 dog teeth (experimental group) after creating an infection and performing cleaning, while no root canal filling was done in the other 50 teeth (control group). The healing progress was outlined concerning the use of the combined material for root canal therapy.

Outline of the experiment

The dogs used in the experiment were more than 1 year old, both male and female, healthy and weighing approximately 5 to 16kg. The mandibles were obtained from 20 heads with 68 back molars and 100 root canals. The composition of KEZ endodontic cement (henceforth KEZ) is shown in Table 1. The gutta-percha points used in this experiment were manufactured by Zipperer.

The technique used in the experiment is as follows. General anesthesia using pentobarbital was administered intravenously. Pre-operative radiographs were taken while the field of operation was left opened by using a diamond cutting tool or abrasive point with iodine and ethanol. Access preparation was done using a #2 or #3 round bur until the roof of the pulp chamber was removed and the pulp cavity was left opened. After a month, radiographs were taken verifying that the root apex was affected. Engine reamers nos. 15-40 were used to perforate the root apex, and hand reamer and K files nos. 40-80 were used to widen the root

Table 1 Components of Endodontic Cement KEZ

[Powder]	%
Zinc Oxide	40.0
Iodoform	20.0
Calcium Hydroxide	20.0
Rosin	20.0
[Liquid]	
Eugenol	90.5
Rosin	9.0
Hardening	0.5

canal. Then, the canals were irrigated with 1% sodium hypochlorite and normal saline solution, and dried with paper points, and complete root canal treatment was done. The first 37 teeth consisting of 50 canals were obturated using combined KEZ and gutta-percha points, followed by phosphoric acid cement and amalgam restoration. This was the experimental group. On the other hand, the next 31 teeth with 50 root canals were not filled and only temporary filling material and amalgam restoration were used. Radiographs were then taken. This was the control group. Three months later, the teeth were extracted from the jaw and subjected to histopathological examination using hematoxylin and eosin stain.

At the time of histopathological examination, detailed observation of the root apex was comprehensively done in all samples regarding tissue organization. The changes observed were recorded using the following criteria. Furthermore, in order to make the criteria more precise, 6 categories and 5 scoring methods were used. In addition to the existing satisfaction system, the inadequacy of score 3 to score 6 was gradually made clear.

1. 5 points–absence of apical periodontitis, regenerative change is characterized tendency to cicatrization, closure of the root apex by calcification can be recognized
2. 4 points–apical periodontitis can be recognized, regenerative change is remarkable
   A score of 4 or 5 implies a good result.
3. 3 points–remnants of apical periodontitis have not yet been resolved but regenerative changes can be recognized, inflammatory change is possibly declining
4. 2 points–remnants of apical periodontitis can still be recognized, the enlarging lesion serves as an obstruction, remnants of apical periodontitis are clear
   A score of 2 or 3 implies a fair result.
5. 1 point–regenerative change cannot be recognized, apical periodontitis is clear
6. 0 points–apical periodontitis is remarkable, regenerative change is not recognizable
   A score of 0 or 1 implies poor result.

Histopathological evaluation

Pathological scores based on the previous criteria are as follow. In the experimental group, 38/50 samples (76%) were recorded to have good results. Among those, 8 samples scored 5 and 30 samples scored 4. Samples 8 and 16 nearly approximated a high satisfactory observation. Moreover, 5 samples obtained a score of 3, and 3 samples obtained a score of 2 for a total of 46 samples (92%) which obtained satisfactory results. Four samples (8%) obtained a below satisfactory score. All those samples got a score of 1, and none of the samples obtained a score of 0.

On the contrary, in the control group, all 50 samples scored below satisfactory, where 18 samples got a score of 1 and 32 samples got a score of 0.

Histopathological evaluation of the experimental group

Figure 1 shows the histopathologic view of the case 17 of the experimental group after a 99 day experimental period. In the mandibular right 2nd premolar, KEZ and gutta-percha points filled the root canal and reached 1.2mm below the root apex. No presumed inflammatory changes existed at that time; the apical tissue was continuous with the periodontal ligament, but cicatrization of the periodontal tissue was remarkable, with various calcifications and soft tissue proliferation. Temporary bone resorption followed by regeneration of adjacent alveolar bone was also observed. In addition, bone resorption and deposition caused by shortening of the root apex was noted. The root apex was not artificially closed but was replaced by cementum. Because of the satisfactory histopathological observation, a score of 5 was given.

Figure 2 shows the histopathological view of case 48, a mandibular left 4th premolar after 91 days experimental period, in which KEZ and gutta-perch point filled the root canal 0.7mm short of the root apex. Residual inflammatory change could still be seen at the root apex. Specifically, inflammatory cell infiltration accompanied by congestion somehow disappeared in the alveolar bone, and hyperplastic granulation tissue was converted to scar tissue. In addition, it was inferred that the scar tissue activated alveolar bone formation. On the other hand, the granulation tissue served as a nidus for the calcification of the root apex, with a tendency for cementum deposition to occur. Furthermore, the alveolar bone directly adjacent became active; soft tissue proliferation induced the calcification of the root apex, and tissue regeneration was remarkable. Therefore, this histological evaluation was given a score of 4.

Figure 3 shows case 14, a mandibular right 4th premolar mesial root after 98 days of experimental period. KEZ and gutta-percha point filled the root canal, showing 0.1mm beyond the root apex. Small residual tissue damage was noted directly underneath the root apex. Specifically, alveolar bone of the root apex was presumed to undergo gradual resorption, cicatrization occurred at the periphery and bone deposition was observed. However, at the part where gutta-percha over extended from the root apex, a small suppuration, congestion and inflammatory cell infiltration still remained, and some granulation tissue and calcified materials were observed. Therefore, this histological observation was given a score of 3.

Figure 4 shows case 4, which is a mandibular right 2nd premolar central root after 98 days experimental period. KEZ and gutta-percha point filled the root canal, which extended by 0.1mm beyond the root apex. The periapical tissues have been seriously damaged. Residual inflammatory cell infiltration was remarkably extensive. However, surrounding the inflammatory tissue, tissue hyperplasia of endothelial cells and alveolar bone regeneration were remarkable. At the root apex, suppuration directly underneath could be recognized, where the tendency toward cicatrization of the periodontal ligament, which is connected to the root apex, showed a tendency to reduce the inflammation. In addition, there was resorption of the root apex, but tissue organization with a tendency to calcify was been observed. Therefore, the regenerative change was observed, and because of the residual inflammatory change, this sample was good, with a score of 2.

Figure 5 shows the histological picture of case 25 with mandibular 1st molar central root after day 99 of the experimental period. KEZ and gutta-percha point filled the root canal, which over-extended by 0.4mm beyond the root apex. Alveolar bone deposition can be recognized, but generally it was without regenerative change. Inflammatory reaction was thought to be present during the entire experimental period. Namely, the periodontal ligament widened, showing

resorption of the alveolar bone with remarkable suppuration and inflammatory cell infiltration directly underneath the root apex at the same time with resorption of the root apex. Therefore, this pathological result was poor, with a score of 1.

Histological examination of the control group

The outline of the typical examples of the control group is as follows. Figure 6 shows the mandibular left 4th premolar mesial root of case 23 after 106 days of experimental period. Root canal filling was not done, and inflammatory condition continued to progress into the alveolar bone without any regenerative change and extended widely into the periodontium with collapsed alveolar bone. Alveolar bone disappeared, and inflammatory nests were filled with young granulation tissue; however, suppuration and remarkable inflammatory cell infiltration were observed with resorption of the root apex. Therefore, this condition was poor and a score of 1 was given.

For case 49, mandibular left 4th premolar mesial root after 91 days of experimental period, root canal filling was not done. There was widening of the periodontal ligament space, and alveolar bone collapsed and disappeared with the existence of pathological change. Namely, there was remarkable suppuration, and inflammatory nests were accompanied by inflammatory cell infiltration with root resorption. Therefore, this sample had poor results with a score of 0.

Figure 7 shows case 4 with mandibular left 3rd premolar mesial root after 99 days of experimental period without root canal filling. Young granulation tissue penetrated the open root apex. In addition, remarkable suppuration and inflammatory cell infiltration were observed in the lower part. Furthermore, there was widening of the periodontal ligament space and bone resorption at the same time there was resorption of the root apex. This histological result was poor and was given a score of 0.

Histopathological evaluation of hemorrhage

Out of 50 samples for each group, 43 samples in the experimental group (86.0%) compared to 39 samples in the control group (78.0%) had congestion. They were divided into three categories: minor ± (Figure 5, point a); mild + (Figure 3, point a); and moderate ++ (Figure 8, point a). Hemorrhage was evident in 2 samples out of 50 (4%) in the experimental group and 2 out of 50 samples in control group (4%), both generally with low ratio. Representative samples of slight ± (Figure 10, point b) and mild + (Figure 9) are shown.

The above characteristics of congestion frequently occur in a large portion showing a minor or mild degree of congestion. This kind of change caused the inflammation of the periapical tissues, or it was thought that the activation of the circulation accompanied the tendency of tissue regeneration. Hemorrhagic change between groups was very slight, and this was thought to be the cause of the healing processes at the beginning, which later on disappeared.

Histopathological evaluation of inflammatory cell infiltration

High inflammatory cell infiltration (Table 2) was observed in the experimental group compared to the control in all cases, but there was a slight degree of difference between the experimental group and the control group. Furthermore, suppuration was categorized into minor ± (Figure 4, point a), minor + (Figure 11, point b), moderate ++ (Figure 5, point b) and strong +++ (Figure 7). In the experimental group, the majority did not show the tendency of the inflammation to enlarge, and it was reduced and limited. In the control group there was a large area of continuous destruction. Originally, in the infected root canals of dog teeth after pulp exposure, it was known that inflammation would always exist, even if there was no apical

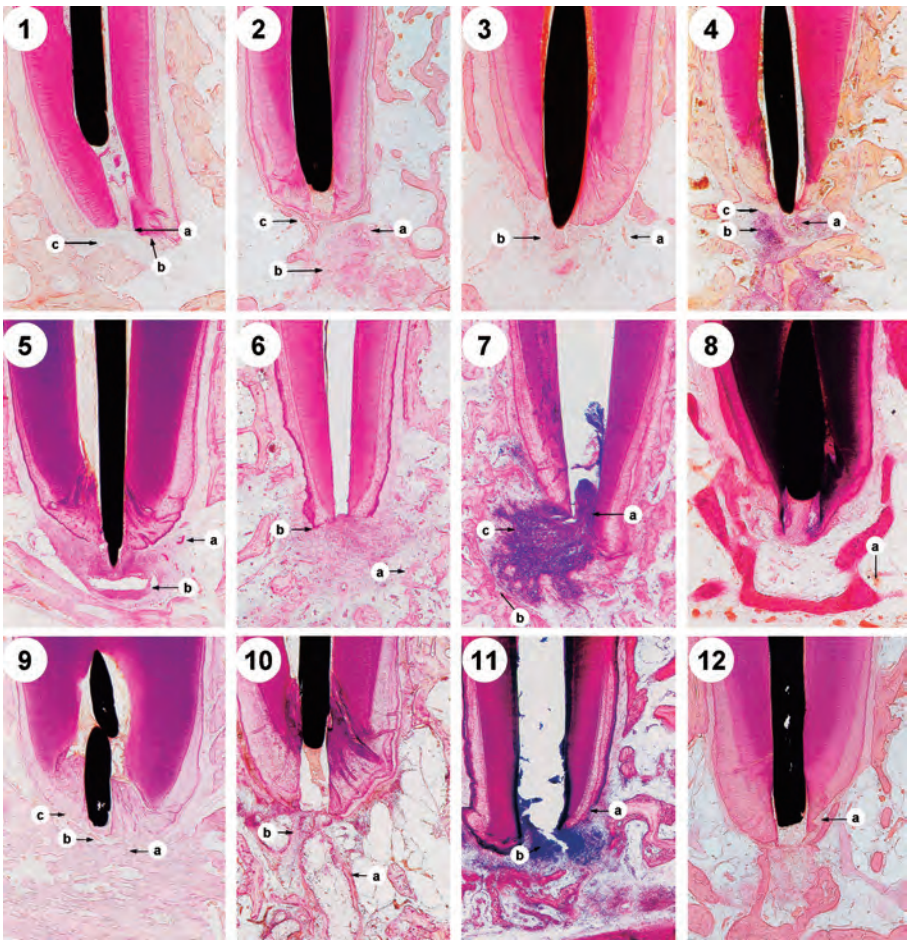


Figure 1 Experimental group, Case 17, Period 99 days, Histopathologically excellent, mandibular right 2nd premolar (a: complete cementum formation; b: apical hard tissue deposition; c: scar tissue).

Figure 2 Experimental group, Case 48, Period 91 days, Histopathologically excellent, mandibular left 4th premolar (a: alveolar bone regeneration; b: inflammatory cell infiltration; c: scar tissue).

Figure 3 Experimental group, Case 14, Period 98 days, Histopathologically good, mandibular right 4th premolar (a: hyperemia; b: scar tissue).

Figure 4 Experimental group, Case 4, Period 98 days, Histopathologically good, mandibular right 2nd premolar (a: superlative inflammation; b: inflammatory cell infiltration; c: scar tissue).

Figure 5 Experimental group, Case 25, Period 99 days, Histopathologically poor mandibular right 1st molar (a: hyperemia; b: superlative inflammation).

Figure 6 Control group, Case 23, Period 106 days, Histopathologically poor, mandibular left 4th premolar (a: alveolar bone regeneration and deposition; b: apical root hard tissue resorption).

Figure 7 Experimental group, Case 4, Period 99 days, Histopathologically poor, mandibular left 3rd premolar (a: root canal polyp formation; b: alveolar bone resorption; c: inflammatory cell infiltration).

Figure 8 Experimental group, Case 27, Period 80 days, Histopathologically excellent, mandibular left 4th premolar (a: hyperemia).

Figure 9 Experimental group, Case 34, Period 125 days, Histopathologically excellent, mandibular left 1st molar.

Figure 10 Experimental group, Case 10, Period 106 days, Histopathologically excellent, mandibular right 4nd premolar.

Figure 11 Control group, Case 21, Period 80 days, Histopathologically poor, mandibular left 3rd premolar.

Figure 12 Experimental group, Case 32, Period 79 days, Histopathologically excellent, mandibular left 3rd premolar.

Table 2 Statement of Varied Lesions Appearing in Apical Periodontal Tissues

Lesions		Hypermia	Hemorrhage	Infiltration	Suppuration	Cicatrix	Apical Absorption	Apical Regeneration	Alveolar Absorption	Alveolar Regeneration	Root Canal Polyp
Experimental (Number)		50									
Expressed Number (%)		43 (86.0%)	2 (40.%)	42 (84.0%)	12 (24.0%)	49 (98.0%)	50 (100%)	47 (94.0%)	50 (100%)	50 (100%)	-
Level	+−	11 (25.6%)	2 (100%)	19 (45.2%)	7 (58.3%)	5 (10.2%)	2 (4.0%)	4 (8.5%)	0 (0.0%)	0 (0.0%)	-
	+	27 (25.6%)	0 (0.0%)	12 (28.6%)	1 (8.3%)	6 (12.2%)	12 (24.0%)	26 (55.3%)	5 (10.0%)	7 (14.0%)	-
	++	5 (11.6%)	0 (0.0%)	8 (19.1%)	4 (33.4%)	6 (12.2%)	12 (24.0%)	17 (36.2%)	31 (62.0%)	34 (68.0%)	-
	+++	0 (0.0%)	0 (0.0%)	3 (7.1%)	0 (0.0%)	32 (65.4%)	24 (48.0%)	0 (0.0%)	14 (28.0%)	9 (18.0%)	-
Control (Number)		50									
Expressed Number (%)		39 (78.0%)	2 (4.0%)	50 (100%)	50 (100%)	26 (52.05)	50 (100%)	0 (0.0%)	50 (100%)	33 (66.0%)	40 (80.0%)
Level	+−	7 (17.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	26 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	17 (51.5%)	18 (45.0%)
	+	27 (69.2%)	2 (100%)	0 (0.0%)	11 (22.0%)	0 (0.0%)	4 (8.0%)	0 (0.0%)	0 (0.0%)	14 (42.4%)	18 (45.0%)
	++	5 (12.9%)	0 (0.0%)	19 (38.0%)	10 (20.0%)	0 (0.0%)	6 (12.0%)	0 (0.0%)	0 (0.0%)	2 (6.1%)	4 (10.0%)
	+++	0 (0.0%)	0 (0.0%)	31 (62.0%)	39 (58.0%)	0 (0.0%)	40 (80.0%)	0 (0.0%)	37 (74.0%)	0 (0.0%)	0 (0.0%)

perforation. At the time of the study, because further penetration to the root apex using a reamer was intentionally performed, the inflammatory process at the apical tissues aggravated the inflammatory changes in all samples. Therefore, in the cases of the control group, where the root canals were not filled, cleaning the canal was not able to resolve the inflammatory process, and heavy inflammatory changes were recognized after 90 days. Compared to the experimental group, where the root canals were filled, 84% still showed residual inflammatory changes and 16% did not show inflammation, though it was inferred that inflammatory changes decreased remarkably. Therefore, it is thought that filling the root canal is an effective way of controlling inflammation.

Cicatrization of the root apex

The capacity of the soft tissue at the lower part of the root apex in the periodontal ligament can be recognized by the transformation of granulation tissue to scar tissue or cicatrization. This kind of change is shown in Table 2. In this regard, in the experimental group, the frequency of scar formation was high while in the control group, activity was shown in the circumference of the inflammatory nests with the formation of granulation tissue; a tendency to repair or to form scar tissue is not recognized. Remodeling of the apical periodontium, soft tissue regeneration, scar tissue formation, and root apex formation were possible. Therefore, an the important objective of this research is to know the pathological changes occurring at the root apex in addition to knowing the effect of the root canal filling system.

Regeneration and calcification of the root apex

Root apex undergoes calcification after root apex resorption exposes the dentin, touching

the granulation tissue; however, regeneration was observed as a pathological view. Also, because tissue regeneration occurred in the resorbed part, the boundary between the regenerated dentin and previous dentin can be recognized. The two groups were almost the same, but more resorption was observed in the control group.

On the other hand, there was regeneration of the root apex after cementum formation at the root apex, or there was dentin resorption. It showed the capacity of the hard tissue formation at the root apex for repair and regenerate. The cells that formed the regenerated cementum tissue were somewhat irregular. However, remarkable tissue regeneration was not found. As for this change, the kind of regenerative change that occurred in the experimental group was different; the majority of cases of regeneration were observed in experimental group; while this kind of regeneration was not observed in the control group. When the regeneration of the root apex was generalized, as in the case of the experimental group, there was hard tissue resorption in all cases compared to the control group. With the experimental group after using the technique, there was advance destruction, and then regeneration was observed after destruction. Therefore, if root canal obturation were not performed, there would be no possibility of restoring the apical tissue.

Closure of the root apex

In root canal therapy, ideal healing is said to be obtained by sealing the root apex, regardless if the root canal is infected. However, this does not depend on the kind or type of root canal filling material while it is in the state of restricted scar formation. As for sealing the root apex, in addition to the cementum material, three other classifications were found: .

i) Cementum substrate formation (Figure 15, point a)

ii) Root apex cementun deposition as a results of apical closure (cementum formation in the area of the defect (Figure 12, point c)

iii) Cementum formation sealing the root apex (Figure 1, point a).

Summary of closure of the root apex due to the addition of new cementum was shown in Table 3.

Namely, in the experimental group of 50 samples, 26, or 52%, were observed to have a closed root apex. On the other hand, this condition was not observed in the control group. Regarding the cementum-like substrate, we reported that in the removal of pulp tissue from the root canal cavity, the fastest formation of the cemental substrate appeared after 41 days, while in the slowest case, 141 days were required for the closure of the apex with cemental substrate, which was considered a long time. Three months after root canal cleaning and obturation in the experimental group, half of the sample had incomplete closure of the root apex and a small number of samples had complete closure of the root apex. Furthermore, if the period had been extended, completion of the root apex might have also been observed in the remaining samples.

Root canal obturation using the combination of zinc oxide and gutta-percha point has been examined before [2, 3, 10] but an examination regarding endodontic cement has not been done. In addition the experiments were done on human teeth without taking into consideration the pathological changes after obturation using zinc oxide cement. Therefore, verification of root apex closure using the new technique was thought to be useful for providing actual proof of success of root canal obturation. It was thought that this kind of healing, where the root apex was not closed, was not observed in the control group because the root canal was not obturated. Inflammation of the apical tissue was followed by the closure of the root apex. Specifically, whether inflammatory change was thought to have occurred, this kind of organization was rarely observed, and when apical periodontitis occurred, tissue regeneration was possible and was then

Table 3 Apical Cementum Formation

	Matrix Only	Irregular Formation	Complete Formation	Total Number
Experimental Group 50 (100%)	8 (30.8%)	13 (50.0%)	5 (19.2%)	26 (52.0%)
Control Group 50 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

followed by closure of the root apex, leading to a remarkable decrease in inflammation (Figure 17). With this kind of case, remodeling of the periodontal membrane close to the root apex would show healing.

Resorption and regeneration of alveolar bone

Resorption of alveolar bone occurs because of inflammation causing bone destruction where a bone cavity is formed. However, from a pathological view point, hyperplasia or regeneration adjacent to resorption can be recognized. The resorption image is outlined in Tables 3 and 4.

As for this change in 50 samples, pathological bone resorption and regeneration was observed in both groups and categorized into 3 groups: mild + (Figure 18, point a); moderate + + (Figure 19, point a); and strong + + + (Figure 7, point b). Both examination groups contained bone resorption, but when compared, the control group had larger bone resorption. When the

Table 4 Distance between Point and Apex

(mm)		Number	Total Number
− 1.6	Excellent	0	1
	Good	1	
	Poor	0	
− 1.5/ − 1.1	Excellent	3	3
	Good	0	
	Poor	0	
− 1.0/ − 0.6	Excellent	8	8
	Good	0	
	Poor	0	
− 0.5/0	Excellent	18	23
	Good	3	
	Poor	2	
+ 0.1/ + 0.5	Excellent	9	15
	Good	4	
	Poor	2	

healing was based on the classification, healing was not immediately observed after 5 days, but relatively resorption occurred 20 days later, and after 25 days, there was cessation of resorption. At the time of the experiment, the root canal was left opened for 1 month, and it was thought that after cleaning of the root canal, further bone resorption occurred in the control group. In the experimental group, bone resorption was controlled. On the other hand, as for bone regeneration, as inflammation declined, repair was initiated and bone deposition at the area of destruction was recognized as bony spicules. The lighter stain of the bone that exists can be recognized as newly-formed bone compared to the pathological bone, and this remodeling phenomenon happened gradually and can be distinguished from the original form. At the same time, as it approaches the root side facing the periodontal ligament, the apical foramen decreases in size. From the above point of view, for all samples in the experimental group, a large portion had moderate to strong bone regeneration. In contrast, the control group had mostly minor changes. This change was thought to be a temporary reaction of the periodontal tissue to bone destruction in which, after a period of time, bone resorption would switch to repair. The tendency for bone regeneration has been mentioned in several reports. In comparison to those reports, it is speculated that the remodeling act completely healed the experimental group compared to the control group, where healing was restricted.

Root canal polyp

When the pulp undergoes necrosis, granulation tissue near the root apex increases, penetrating the root canal, and this condition is generally known as a root canal polyp. The formation of a root canal polyp is of 3 kinds: (1) endothelial cell; (2) scar tissue; (3) root canal osteoid. Types 2 and 3 suggest healing. In this experiment (Table 1), it was not recognized in 50 samples in the experimental group. In contrast, it was recognized in the control group in 40 samples (80%) (Figure 7, point c). From the above fact, it was thought that in the experimental group, because the canal was filled with gutta-percha point and cement without an air gap, bleeding did not occur within the root canal. On the other hand, in the control group many samples revealed inflammatory granulation tissue to the extent that there was scar and pulp polyp formation. The root canal being empty is the reason why pulp polyp formation easily occurred.

Over- and short-root canal obturation

Root canal obturation generally uses a gutta-percha point which can be classified as flush (normally, the root apex is located 0.5mm short or exactly approaching the border zone of the root canal constriction), short (the root canal filling does not terminate to the zone of root canal constriction), or overextended (obturation beyond the root apex, excess filling material). The connection to healing is discussed in relation to the kind of obturation. Namely, there are various opinions that bring about satisfactory healing under obturation [7, 10-12], the opinion that under obturation is bad healing[13-16], that flush brings satisfactory healing [3], flush brings bad healing [11], overextended brings good healing [17], or overextension is defective [11, 12]. Therefore, these opinions are not easy to judge. However, the main discussion has focused on x-ray images and the pathological view has barely been discussed [10]. Clinically, it is better to say that the actual anatomical position of the apex is just a ‘measure’ and that the exact reference cannot be located. In addition, in case of combined use of root canal obturation (endodontic cement), the location becomes an object of discussion, although this point has not clearly been expressed. Furthermore, it is expected that the general position of the root canal constriction is not distinct. In this experiment, because the root apex was artificially formed in dog’s teeth,

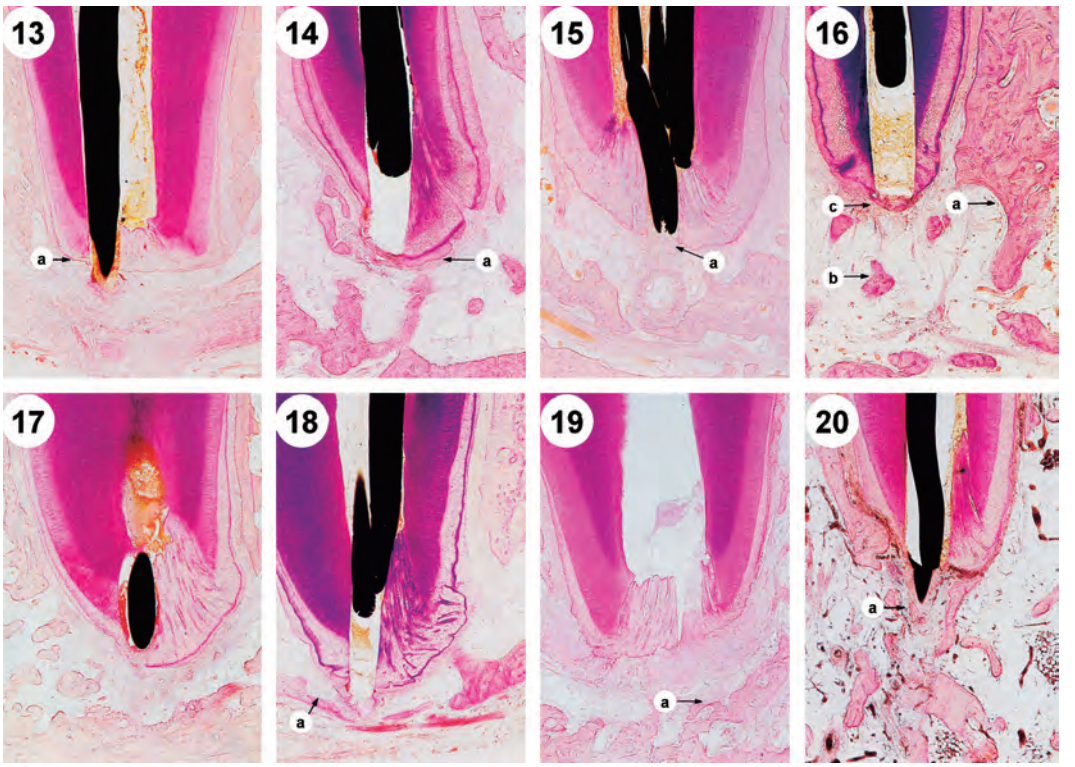


Figure 13 Experimental group, Case 50, Period 91 days, Histopathologically excellent, mandibular left 1st molar.
Figure 14 Experimental group, Case 42, Period 91 days, Histopathologically excellent, mandibular left 4th molar.
Figure 15 Experimental group, Case 16, Period 98 days, Histopathologically excellent, mandibular right 1st molar.
Figure 16 Experimental group, Case 43, Period 91 days, Histopathologically excellent, mandibular left 4th premolar.
Figure 17 Experimental group, Case 21, Period 99 days, Histopathologically excellent, mandibular right 1st molar.
Figure 18 Experimental group, Case 44, Period 91 days, Histopathologically excellent, mandibular left 1st molar.
Figure 19 Experimental group, Case 17, Period 99 days, Histopathologically poor, mandibular left 1st molar.
Figure 20 Experimental group, Case 1, Period 99 days, Histopathologically excellent, mandibular right 3rd premolar.

proper constriction does not exist, but the root apex and position in relation to the combined use of root canal filling could be measured clearly. Namely, Table 4 shows the relationship between the gutta-percha point and the root apex. When the pathological report was checked, the obturation observed to be flush was moderate, those that were short were more satisfactory and those that were overextended were inferior. Therefore, from the result of this experiment, short gutta percha (0.6-1.6mm) is superior, next is flush (0-0.5mm from the apex) and the last is overextended (0.1-0.5mm beyond the apex). Cleaning and immediate obturation with the combined use of KEZ and gutta perch-point done in short obturation is the superior technique in our opinion. However, because it is a system of combined use for root canal obturation, the location of the gutta percha point whether it is adequate or not to affect healing is still doubtful. It

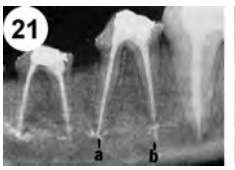
is likely that KEZ, whether it is underfilled or overextended, would influence the pathological condition. First, regarding the insufficient obturation, KEZ barely exceeded the root apex which was seen in the pathological view. On the other hand, gutta-percha point filled excessively outside the root apex in case 15, and KEZ barely has come in contact around the gutta-percha point in one case (Figure 13) as it was observed to be restricted. In another 14 cases, the adhesion of KEZ was not recognized. On the other hand, in the radiographs immediately after obturation, in 32 cases out of 50 cases KEZ was observed to spread excessively beyond the root apex, although after 3 months KEZ was not recognized at the root apex in the cases, suggesting that KEZ has been resorbed and thus disappeared. Therefore, whether KEZ could cause inflammation and resorption is unclear after 3 months, but KEZ showed superior characteristics as an endodontic cement because it would stay inside the root canal while materials located outside would undergo resorption with a healing tendency of the periapical tissues. On the other hand, regarding the overextended obturation, 15 cases showed limited healing tendency, and remarkable inflammatory changes, and residual inflammation could still be observed around the gutta-percha point (Figure 5). The remaining 8 of 15 cases showed minor inflammatory changes. In the other 7 cases there had been soft tissue organization of the apical tissues with scar tissue formation (Figure 20, point a).

Root apex and apical tissue regeneration

The essence of this research is provide a detailed pathological view comparing the experimental and control groups regarding the resorption of alveolar bone and regeneration and scar tissue formation with regard to the usefulness of the technique. However, the root apex that stimulates the remodeling of the apical tissues was not pointed out and was not explained. It goes without saying that healing after obturation of the root canal is assumed with closure of the root apex that is desirable for healing of the apical tissues. Originally, the material used to seal the root canal was thought to block biological healing, along with the technique of combined use of root canal obturation to seal the apex; however, tissue regeneration was ascertained. Even with the addition of resilience to root apex closure, inflammatory changes in bone tissue destruction and deep tissues would remain for a long period of time and have a tendency to progress, allowing symptoms to spread into the periapical tissues. Therefore, the crucial issue is whether the alteration has a tendency to develop inflammatory changes and bone repair. In this regard, by comparing the experimental group with the control group, it has been confirmed that the observed changes in repair sometimes showed a greater tendency to delay the disappearance of inflammatory lesions than those at the root apex. With the proliferation of granulation tissue from surrounding scar formation, newly-formed vessels where inflammatory lesions develop were frequently observed, followed by bone scarring added to the center of bone regeneration. In this study, it seemed that periodontal tissues were completely restored and relatively small opened root apeces were observed, yet many still were expected to recover during the experimental period.

KEZ as root canal filling material and x-ray contrast

X-ray examination after root canal obturation aimed to examine the following: 1) the closed state of the root canal filling material; 2) sealed root apex; 3) overextension of the root canal filling material from the root apex; and 4) the apical tissues. From the standpoint of the materials used in root canal treatment, such material should seal the root canal cavity for a long time and should remain radiographically visible (radiopaque). KEZ applied in this experiment contains iodoform and a large ratio of zinc oxid, making it clearly visible in radiographs. In addition,



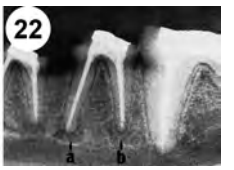


Figure 21 Penetrating endodontic cement KEZ just after the root canal filling. a: Case 42: mesial root; b: Case 43: distal root both in Experimental group, mandibular left 4th premolar.

Figure 22 Radiograph of 3 months after the root canal filling show the disappearance of the KEZ. a: Case 42: mesial root; b: Case 43: distal root both in Experimental group, mandibular left 4th premolar.

because the gutta-percha point and the carrier of the endodontic cement have a different contrast, root canal obturation can be seen clearly. Figure 21 shows a number of cases of root canal performed after surgical procedure. The mesial root of the mandibular left premolars from 4 foramina where the root tip is 1.0mm, the 4 distal roots of mandibular left premolars are just 1. 2mm gutta percha point, and there was a slight extravasation of KEZ sealant from the root tip. Three months later, a radiograph of the same site (Figure 22) was taken and the extravasation at the root apex was not seen. Figure 14 shows the tissue sample, and in Figure 16, the shape of the cement is no longer visible after 3 months. However, in Figure 14, root canal cavity at the end of the apex is hollow, but this is in Figure 16, which is an artifact of residual cement where both cements remained in the root canals. Typical examples shown here had large extravastion of the cement, but 3 months later, regardless of the amount of extravastion, the residual cement was resorbed in most cases, and in 32 cases only 3 had an insignificant amount of residual cement. From the results, KEZ is effective in binding together gutta-percha points, and even if extravasation of the cement to some extent into apical tissues will occur, resorption would also occur. In order to prove the existence of such endodontic cement, it was placed side by side with barium sulfate [18]. X-ray images with barium sulfate were good and the material was reported to be non-cytotoxic [12, 38], suggesting that when the materials are placed side by side, the iodoform endodontic cement provides a good solution.

Summary of the pathological evaluation and discussion

The pathological result of this study is summarized on Table 5. Specifically, in the experimental group, 38 out of 50 samples or 76.0% were evaluated to have good healing. Out of the 38 cases, 8 cases received a score of 5, and 30 cases a score of 4. In addition, 8 out of 50cases, or 16.0%, were evaluated to be good; 3 cases got a score of 3; and 5 cases got a score of 2. A total of 92.0% (46 cases) achieved satisfactory results and only 4 cases or 8.0% were poor, and when evaluated, all 4 cases got a score of 1 and none got a score of 0. On the other hand, in the control group, all 50 samples got poor results, where 18 cases got a score of 1 and 32 cases got a score of 0. The above results showed that the experimental group was significantly better when compared to the control group. In experimental studies on root canal filling using infected canals of animals we performed 2 to 3 cleanings and disinfections for root canal treatment. We

Table 5 Summary of Histopathological Examination

	Number	Result					
		Excellent		Good		Poor	
		5	4	3	2	1	0
Examination Group	50	8	30	5	3	4	0
		38 (76%)		8 (16%)		4 (8%)	
		46 (92%)					
Control Group	50	0	0	0	0	18	32
		0		0		50 (100%)	
		0					

not only induced infection of the root canal but an artificial apex was created. Comparing our results with those of previous experiments is not appropriate since the experimental conditions are not the same. In this experiment, cleaning of the canal was not immediately done compared to previous studies and so results could be inferior or superior. The ability of the combined use of KEZ and gutta-percha point to seal the root canal is not only valid but may also stimulate the periapical tissues against persistent and harmful agents bringing out the body's ability for repair. When the findings obtained from experimentally-infected root canal treatment in dogs are clinically applied, we must pay attention to the time difference of the biological reaction between dogs and humans [2, 39]. The results of this study suggest that the actual dental root canal treatment method can be used.

Conclusions

The authors used dog teeth obtained from 19 mandibles consisting of 68 teeth with 100 root canals. Root canal enlargement was done, and once clean, 50 root canals were immediately filled with KEZ and gutta-percha points as the experimental group, and 50 canals were closed at the level of the pulp chamber without filling the canal. Histopathological examination was done 3 months after surgery. The results were as follows:

1) In the control group, bone regeneration and scarring changes quite slowly to the extent that mild or minor scarring around the inflammatory foci indicating granulomatous formation or tendency to repair or scarring was not observed. Alveolar bone regeneration and the defense of the body show a feeble response. There was no soft tissue closure, which is the ideal form of healing after root canal therapy and hard tissue regeneration. The disappearance of inflammation could not be expected, and inflammatory changes were severe in all patients after 3 months. This suggests that healing is not expected after only root canal cleaning and closing of the pulp chamber, and this also demonstrates the need for root canal obturation.
2) Periodontal inflammatory changes at the root apex in the experimenat group showed 84% healing, and only 16% had some degree of inflammation or no change, and even the remaining cases had a significant tendency to decrease in inflammation. Therefore, the decrease in inflammation using this system in root canal treatment is noteworthy.
3) Scarring as remodeling of the periodontal tissue has a higher frequency in the experimental group, showing the efficacy of the root canal system.

4) Hard tissue regeneration of the root apex was added as a kind of healing after a period of time in the experimental group, and the progression of the lesion ceased, as well as the destructive changes.
5) In the experimental group, complete closure of the root apex with regeneration and hard tissue substrate formation were observed in approximately half of the cases, although progression in the remaining cases could still occur afterward. There was closure of the root apex with deposition of hard tissue, loss of bony defects, and a tendency for soft tissue regeneration and repair around the periodontal ligament.
6) In the experimental group, bone regeneration was seen in all cases, even in those with moderate or intense alveolar bone defects; bone regeneration started from the edge of the alveolar bone defects and was complicated by the tendency to restore bone trabeculae which were once destroyed, but was confirmed to be completely repaired with oriented remodeling and repair.
7) When used with root canal treatment, the degree of obturation can be shown in terms of frequency, as follows: (a) short (0.6-1.6mm) is the highest; (b) flush (0-0.5mm from the root apex) is next; and (c) overextended (0.1-0.5mm beyond the root apex).
8) The endodontic cements used in the experiment have good x-ray contrast. They are effective for record of (1) obturation material in root canal treatment, (2) sealing of the root apex, and (3) sealing condition of the root apex, because of the confirmation of maintain the contrast for a long period of time.
9) Pathological evaluation of the study showed that in the experimental group, 38 out of 50 samples (76%) were good, 8 of the samples got a score of 5, and 30 samples got a score of 4. In the other 8 samples (16%), 5 samples got a score of 3, and 3 samples got a score of 2. If both results are added, the total satisfactory result amounts to 46 samples (92%), with only 4 cases (8%) below satisfactory (all 4 cases got a score of 1 and none of the samples got a score of 0). In the control group, those with a poor pathological evaluation were 18 cases, which received a score of 1, and 32 cases, with a score of 0.
10) Therefore, it was found that the combination of KEZ endodontic cement and gutta-percha point as root canal filling material showed moderate x-ray contrast, had the ability to seal the root canal, did not irritate the periodontal tissue, and promoted healing, ensuring the reliability of this method.

References

[1] Gottlieb B, Orban B and Stein G (1933) Die Wurzelbehandlung bei lebender Pulp. Z Stomat 31(10): 665-681.
[2] Dixon CM and Rickert UG (1938) Histologic verification of results of root canal therapy in experimental animals. J Amer Dent Ass 25: 1781-1803.
[3] Buchbinder M (1936) A statistical study of root canal therapy. Dent Cosmos 78: 20-26.
[4] Jasper EA (1941) Adaptation and tissue tolerance of silver root canal fillings. J Dent Res 20: 306-355.
[5] Ingle JI (1956) Root canal obturation. J Amer Dent Ass 53: 47-55.
[6] Grossman LI (1964) Roentogenologic and clinical evaluation of endodontically treated teeth. Oral Surg17: 368-374.
[7] Davis MS (1971) Periapical and intracanal healing following incomplete root canal fillings in dogs. Oral Surg 31: 662-675.
[8] Baker BCW (1972) Reaction of dog tissue immediate root canal filling with zinc oxide cement and gutta-percha. Aust Dent J 17: 1-8.
[9] Binnie WH and Rowe AHR (1973) A histological study of periapical tissues of in completely formed pulpless teeth filled with calcium hydroxide. J Dent Res 52: 1110-1116.
[10] Boyle PE (1955) Kronfield's Histopathology of the Teeth and their Surrounding Structures. Lea and Febiger,

Philadelphia.
[11] Seltzer S, Bender IB and Turkenkopf S (1963) Factors affecting successful repair after root canal therapy. J Amer Dent Ass 67: 651-662.
[12] Harty FJ, Parkings BJ and Wengraf AM (1970) Success rate in root canal therapy. Br Dent J 96: 65-70.
[13] Coolidge ED (1960) Past and present concepts in endodontics. J Amer Dent Ass 61: 676-688.
[14] Ingle JL (1961) A standardize enendodontic technique utilizing newly designed instruments and filling materials. Oral Surg 14: 83-91.
[15] Grossman LI (1974) Endodontics: A peep into the past and the future. Oral Surg 31: 599-608.
[16] Grove CJ (1929) An accurate new technique for filling root canals to the dentinocemental junction with impermeable materials. J Amer Dent Ass 16: 1594-1600.
[17] Ottesen I (1937) Die Behandlung der Granulome durch den Wurzelkanal. Z Stomat 35: 552-561.
[18] Cohen S and Burns RC (1976) Pathways of the Pulp. C. V. Mosby Company, Saint Louis.

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